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PROJECT NUMBERS
NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

Z01 AR 27000-31 LPB

Z01 AR 27001-19 LPB

Z01 AR 27002-15 LSBR

Z01 AR 27003-34 LPB

Z01 AR 27004-24 LPB

Z01 AR 27005-11 LPB

Z01 AR 27012-09 LPB

Z01 AR 27013-02 LSBR

Z01 AR 27014-02 LPB

Z01 AR 41022-21 ARB

Z01 AR 41023-19 ARB

Z01 AR 41025-22 ARB

Z01 AR 41040-21 ARB

Z01 AR 41048-14 ARB

Z01 AR 41066-11 ARB

Z01 AR 41074-06 ARB

Z01 AR 41076-06 ARB

Z01 AR 41083-04 ARB

Z01 AR 41084-04 LSB

Z01 AR 41085-04 LSB

Z01 AR 41086-04 LSB

Z01 AR 41087-04 LSB

Z01 AR 41088-03 ARB

Z01 AR 41089-02 LSB

Z01 AR 41090-02 LSB

Z01 AR 41091-02 LSB

Z01 AR 41092-02 ARB

Z01 AR 41093-02 LSB

Z01 AR 41094-02 LSB

Z01 AR 41095-02 ARB

Z01 AR 41099-02 ARB

Z01 AR 41100-01 ARB

Z01 AR 41102-01 ARB

Z01 AR 41103-01 ARB

Z01 AR 41104-01 ARB

INACTIVE PROJECTS

Z01 AR 41096-02 ARB

Z01 AR 41097-02 ARB

Z01 AR 41098-02 ARB

RC
925
N277
1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27000-31 LPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J. Podolsky	Chief	Muscle Biophysics Section, LPB, NIAMS
	Shigeru Chaen	Visiting Scientist	LPB, NIAMS
	Yutaka Sasao	Visiting Associate	LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. Robert Horowits, Section on Muscle Biophysics, LPB; Dr. Alasdair Steven, LSBR, NIAMS; Dr. Ellis Kempner, Section on Macromolecular Biophysics, LPB; Dr. Neal Epstein, NHLBI; Dr. Lameh Fananapazir, NHLBI.

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Titin is required for lateral stability of the myosin filament lattice in relaxed fibers. The spacing of nearest-neighbor myosin filaments in electronmicrographs of cross sections through the A band was measured in irradiated muscle fibers in the relaxed and rigor states. The irradiation dose was selected to degrade the titin molecules but leave the smaller molecules intact. In relaxed fibers at 20 degrees C, the average filament spacing was the same in irradiated fibers and controls, but the standard deviation of the control fiber spacing distribution was smaller. This result is evidence that the presence of intact titin promotes lateral order in the array of myosin filaments. In rigor fibers the standard deviation of the spacing distribution was the same in irradiated and control fibers, showing that actomyosin crossbridges also stabilize the myofilament lattice.

2. Properties of mutant myosin in muscle fibers from patients with familial hypertrophic cardiomyopathy (FHC). The contractile force, the unloaded contraction velocity, and the ATPase activity were measured in calcium activated, skinned soleus muscle fibers in control fibers and in mutant 741. Force and velocity were down two to three fold in the mutant, but ATPase activity was nearly doubled. It is concluded that in the mechanical and chemical activities of the myosin molecule in the mutant are less tightly coupled than in the normal fiber.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27001-19 LPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle Contractility and Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Mark Schoenberg Medical Officer LPB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Physical Biology

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In FY 93 our study of the myosin crossbridge interaction with actin in muscle fibers focused on the differences between the weakly-binding and strongly-binding crossbridge interactions with actin. The hypothesis was formulated that the major difference between weakly- and strongly-binding crossbridges is that the heads of the former are mobile after attachment whereas those of the latter are not. This explained almost all of the known differences in behavior between weakly- and strongly-binding crossbridges, including why the kinetics of weakly-binding crossbridges are not independent of ionic strength while those of strongly-binding crossbridges are not. It also explained why weakly-binding crossbridges relax tension about as quickly as myosin subfragment-1 detaches from actin in solution, but strongly-binding crossbridges, under most conditions, do so much more slowly. The molecular structural properties of myosin important in determining whether it binds weakly or strongly were investigated by examining the reaction sites for N-phenylmaleimide, a compound which, when it reacts with myosin, locks it in the weakly-binding configuration. It was found that N-phenylmaleimide alkylates myosin heavy chain at Cys-697 and Cys-707.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27002-15 LSBR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Biology of Macromolecular Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alasdair C. Steven, Chief, Laboratory of Structural Biology Research/NIAMS
Others:

Frank Booy, Visiting Scientist - Jose Caston, Special Volunteer
Mario Cerritelli, IRTA Fellow - Naiqian Cheng, Visiting Associate
James Conway, Visiting Associate - Eva Kocsis, Visiting Associate
Manoj Misra, Visiting Associate - Benes Trus, Guest Researcher

COOPERATING UNITS *(if any)*

Computer Systems Lab, Div. Computer Res. & Tech., NIH; Dept. of Microbiology, Univ. of Virginia. Dept. of Biology, Brookhaven Nat'l Lab, (Drs. J. Wall, J. Hainfeld); others as noted.

LAB/BRANCH

Laboratory of Structural Biology Research

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
9.2	8.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Laboratory seeks to elucidate the mechanisms that govern the assembly of supramolecular complexes and the folding of macromolecules, as well as those that underlie the synthesis of viruses, organelles, and other high order structures. In the past year, we have discovered a radical departure from equivalence in the packing of protein molecules in the capsid of bacteriophage HK97. The same protein forms both the hexons and pentons, but whereas the pentons are cyclically symmetric pentamers, the hexons are not cyclically symmetric, but consist of two apposed trimers with a mutual lateral displacement of at least 20 Angstroms. In herpes simplex virus, we have localized the small abundant capsid protein, VP26 (12 kDa) to the outer tips of hexons by quantitative difference imaging. Its exposed location suggests that its role may lie in coupling the capsid to the surrounding tegument. We have also studied several of the major molecules displayed at the outer surface of the pathogenic bacterium, Bordetella pertussis. One of these, filamentous hemagglutinin, is a high immunogenic adhesion. FHA was found to assume a novel 50nm-long monomeric hairpin structure. Its sequence contains two protracted runs of 19-residue repeats which strongly resemble the leucine-rich repeats (LRRs) that are present in a set of eukaryotic proteins, many of which have been implicated in intercellular adhesion phenomena. This observation suggests that FHA repeats may be involved in the adhesion of B. pertussis cells to the respiratory tract.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27003-34 LPB

PERIOD COVERED

October 1, 1992 to September 20, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ellis S. Kempner, Ph.D. Physicist and Chief,
Macromolecular Biophysics Section, LPB, NIAMS

COOPERATING UNITS *(if any)*

Drs. T. Boyer (Emory); B. Fleischer (Vanderbilt); E.H. Harrison and A.C. Ross (Pennsylvania Medical College)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Macromolecular Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Target analysis of the inactivation of biological activity by ionizing radiation was applied to several major problems including cocaine receptors in brain, various enzymes, and energy transfer between synthetic polymers.

Objectives: 1) An understanding of the nature of active structures *in vivo* which are involved in biochemical processes, principally by means of the technique of inactivation by ionizing radiation. 2) Detailed knowledge of the molecular damage caused by ionizing radiation and of the mechanisms of the transfer of radiation energy throughout these structures.

Methods: 1) General biochemical techniques including enzyme reactions, fluorescence, and gel electrophoresis. 2) Ionizing radiation, usually high energy electrons from a linear accelerator, to expose samples under carefully controlled conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27004-24 LPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Norman Gershfeld, Ph.D.	Research Chemist	LPB, NIAMS
Others:	Kazuo Tajima, Ph.D.	Visiting Scientist	LPB, NIAMS
	Kazuhiro Fukada, Ph.D.	Visiting Fellow	LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. Courtney P. Mudd, ACES, BEIP, NCRR

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Macromolecular Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The configuration of lipid in membranes as a single bilayer has been shown to be a unique state that can self-assemble only at a critical temperature, the physiological temperature of the cell, and with a critical composition that reflects metabolic processes within the membrane and cytosol of the cell. The principles which govern the conditions for assembly of the critical unilamellar state of the membrane has been the primary focus of this project. We have applied these principles to Alzheimer's disease brain tissue, and have observed a lipid defect in membranes that is tissue specific. During this past year we have developed a new calorimetric method that is generally applicable for measuring the critical temperature for membrane assembly, and for specific chemical contributions to stability of membrane bilayers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27005-11 LPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aggregation of Human Platelets Induced by Decompression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Makio Murayama Research Chemist LPB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Physical Biology

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main thrust of the laboratory is focused on the molecular mechanism of platelet aggregation, including both hemostasis and platelet aggregation inducible by decompression (both by reduced barometric and hydrostatic pressure). We are investigating the influence of change of pressure on the hydration layer surrounding the electrically charged amino acid residues involved in platelet aggregation. We have been investigating the molecular mechanism of platelet interactions in DIPA (decompression-inducible platelet aggregation) for the past several years. We have found that oppositely charged amino acid residues interact with their respective receptor sites. We have observed vascular occlusion in the small blood vessels in the web of the frog's foot and in the ear of the mouse. During the current year we have confirmed that the volume of human PRP is increased by 0.3% or 3 ml per liter of packed thrombocyte volume when platelet aggregation is induced by decompression. A similar volume increase has been observed when platelet aggregation is induced by the agonist, epinephrine (adrenalin), ADP, collagen and PAF (platelet activating factor). We theorize that compactly organized water molecules, when randomized into the bulk phase, acquire thermal motion which causes plasma volume to increase and a temperature drop; and that human platelet aggregation is an entropy driven process similar to human red cell sickling. To confirm our hypothesis, we are continuing our experiments with a specially designed dilatometer, including a thermistor, to measure the volume increase and temperature drop when platelet aggregation is induced by the agonists epinephrine, ADP, and PAF. During the year we have obtained additional confirmation of volume increase of human plasma due to decompression by using the discontinuous, density gradient zonal centrifugation method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27012-09 LPB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Mechanical Properties of Muscle Fibers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leepo C. Yu	Research Physicist	LPB, NIAMS
Others:	Sengen Xu	Visiting Associate	LPB, NIAMS
	Sergey Malinchik	Visiting Associate	LPB, NIAMS
	Suzanne Frisbie	IRTA	LPB, NIAMS
	Dan Gilroy	Mathematician	LPB, NIAMS

COOPERATING UNITS *(if any)*

Medical School of Hannover, FRG (Drs. B. Brenner and T. Kraft); East Carolina University Medical School, North Carolina (Dr. J. Chalovich).

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To understand muscle contraction at molecular level, we (i) identify in vivo structural changes that are associated with reaction steps within the actomyosin (cross-bridges) ATPase cycle; (ii) characterize the nature of these structural differences; and (iii) characterize mechanical properties of different actomyosin complexes in muscle.

Under a wide range of conditions, response of the contractile system to osmotic compression can be used to screen for structural and mechanical differences of attached cross-bridges. In FY 92-93 we found that both the structure and elastic properties of the weakly attached cross-bridges are distinct from active crossbridges or cross-bridges in rigor. This is significant since the weakly attached cross-bridges represent the pre-force generating states. Our findings further strengthened the hypothesis that force generation results from a structural transformation from a weakly attached to a strongly attached configuration -- a hypothesis supported by the recently published crystal structures of actin, the myosin head and decorated actin filaments.

Molecular structure of demembranated relaxed psoas muscle fibers at low temperature is being studied by two dimensional X-ray diffraction. Under these conditions, large fraction of cross-bridge are attached in the pre-force generating states. The diffraction patterns were found to consist of myosin layer lines without rigor features. The meridional intensities at 4400 nm and 2150 nm are approximately 5 times stronger that the 1430 nm reflection, a feature not observed previously in any other preparations. The present results suggest that attachment of pre-force generating cross-bridges shares few if any features with that of the cross-bridges in rigor and distribution of cross-bridges is repeatedly perturbed from the regularly spaced 143 nm levels along the thick filament. The significance of the finding is that even with the atomic structures of actin and myosin fragments becoming known, for any modelling of force generation in muscle, it will be necessary to have information of mass distribution along the filament axis such as those derived from in vivo two dimensional X-ray diffraction studies.

Saturation of cross-bridges with nucleotide analogs ATP gamma S and GTP were found to vary over a wide range of concentrations. With modelling, these data should yield information on the elastic properties of a single attached myosin head, a central yet so far unknown quantity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27013-02 LSBR

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High-Resolution Structure and Function of Biological Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Craig Hyde	Expert	LSBR/NIAMS
Others:			
	Steven L. Edwards	Sr. Staff Fellow	LSBR/NIAMS
	Joseph P. Mack	Sr. Staff Fellow	LSBR/NIAMS
	Vaidehi Sridhar	IRTA Fellow	LSBR/NIAMS

COOPERATING UNITS *(if any)*

ARB/NIAMS (Paul Plotz); LBP/NIDDK (Nancy Nossal, Reed Wickner, William Coleman); DCBDC/NCI (Michael Maurizi, Joan Hanley-Hyde); BRB/NIAID (John Coligan); PEL/OD (Paul Wingfield, Stephen Stahl).

LAB/BRANCH

Laboratory of Structural Biology Research

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major interest of the x-ray crystallography working group, established last year within the Laboratory of Structural Biology, is broadly based in the study of the structure and function of macromolecular assemblies and multienzyme complexes. Other areas of interest within the group involve basic research on the structure of retroviral proteins and host cellular factors involved in HIV expression.

The group is now near full strength and is well equipped with instrumentation and equipment. We have equipped ourselves with a modern x-ray diffraction data collection system, high-performance computer workstations, computer-graphics workstations, and facilities for preparative-scale expression and purification and crystallization of proteins.

The group has spent a great deal of effort to obtain large amounts of pure proteins and macromolecular complexes and to obtain crystals suitable for high resolution x-ray diffraction studies. Protein expression studies of several cyclins are underway. We can now reliably produce large quantities of pure, soluble forms of human Nuclear Factor K-B (NFkB), the human histidyl tRNA synthetase, and retroviral intergrase. Several collaborators have demonstrated an ability to provide us with the quantitites and purity of proteins needed for our structural studies. Small, but promising, crystals of whole LA viral particles, the "CLP-A" ATP-binding/protease regulatory subunits have been grown. Large, diffraction quality crystals of a bacterial phosphoribosyl transferase, and protease complex, human HLA Class 1/peptide complexes and the tRNA synthetase have been obtained. Full structure determinations are now underway. Three of these four structures will likely require us to use the more complicated and lengthy methods of multiple heavy-atom isomorphous replacement. All of these projects are complicated for a variety of technical reasons including the mechanical and radiation stability of the crystals or large unit cell parameters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27014-02 LPB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Myofibril Assembly and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Horowits, Ph.D.	Research Biologist	LPB, NIAMS
Others:	Gang Luo, Ph.D.	Visiting Fellow	LPB, NIAMS
	Jin Qiao Zhang, Ph.D.	Visiting Fellow	LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. Richard Podolsky, NIAMS; Dr. Neil Epstein, NHLBI; Dr. Lameh Fananapazir, NHLBI; Dr. James R. Sellers, NHLBI

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As a first step to studying the function of extremely large myofibrillar proteins using molecular genetic approaches, genes encoding these proteins must be isolated and cloned. In an attempt to isolate a clone carrying large portions of the coding sequence for mouse nebulin, a cDNA library was constructed using conditions designed to optimize the chances of cloning very large cDNAs. A putative nebulin cDNA clone has been isolated from this library. The clone carries an insert approximately 18 kilobases in length. Control experiments indicate that the entire insert in uncontaminated by the vector sequence, and hence must be genuine cDNA. We are currently mapping the insert for restriction endonuclease sites, and subcloning and sequencing selected segments.

Several distinct mutations in the beta-myosin heavy chain (MHC) gene have been linked to hypertrophic cardiomyopathy (HCM), a serious genetic disease of the heart. Because the cardiac beta-MHC is also expressed in slow-twitch fibers of skeletal muscle, we have been able to study the mechanical properties associated with these myosin mutations in single skeletal muscle fibers obtained from HCM patients. We found that the normal and the mutant copy of the beta-MHC gene are on average equally expressed within single slow-twitch skeletal muscle fibers. Furthermore, we found that three distinct missense mutations in the beta-MHC gene that cause HCM produce either no change or only a small decrease in isometric force output of single slow-twitch skeletal muscle cells. In summary, these results demonstrate that isometric force production by myosin mutants need not be affected to cause HCM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41022-21 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Studies in Murine Lupus.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry Metzger, M.D.	Acting Chief, Cellular Immunology Section	ARB/NIAMS
	Dorothy Scott, M.D.	Special Volunteer	ARB/NIAMS
	Wendy Kisch	Biologist	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Disease in lupus-prone mice is dependent upon T cells. Many T cells phenotypic abnormalities have been described in lupus-prone mice. One possible mechanism for development of autoimmunity-promoting T cells is failure to appropriately delete self-reactive cells. To study peripheral deletion and anergy, peripheral T cell deletion was studied in lupus-prone strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41023-19 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Patients with Immune-mediated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry Metzger, M.D.	Acting Chief, Cellular Immunology Section	ARB/NIAMS
	Mark Gourley, M.D.	Special Volunteer	ARB/NIAMS
	Wendy Kisch	Biologist	ARB/NIAMS
	Geryl Wood	Biologist	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with Systemic Lupus Erythematosus have an increased frequency of HPRT mutated T cells which allow the cells to grow in a selective medium of 6-thioguanine. Multiple clones from SLE patients and normal subjects have been isolated and cellular characteristics have been characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41025-22 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Cell Surface Receptor for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Henry Metzger, MD Chief, Chemical Immunology Section ARB/NIAMS

Su-Yao Mao, PhD	Visiting Associate	Clara Pribluda, MD	Visiting Fellow
Carole Jelsema, PhD	Senior Staff Fellow	Becky Vonakis, PhD	IRTA
Ute Kent, PhD	IRTA Fellow	Jorge Gomez, MD,PhD	IRTA
Victor Pribluda, PhD	Visiting Associate	T Yamashita, PhD	Visiting Fellow
Lisa Rider, MD	Clinical Associate	Hana Smith	Chemist
Juan Rivera, PhD	Senior Staff Fellow	Dr. Carla Wofsy	IPA
Patrizia Germano, MD	Visiting Fellow	Dr. Byron Goldstein	IPA

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Chemical Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N258, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
13.5	12.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The high affinity receptor for IgE on mast cells and basophils (FcEpsilonRI) plays a central role in immediate hypersensitivity reactions. Aggregation of receptor-bound IgE by polyvalent antigen leads to aggregation of the receptors and cellular secretion of both preformed and newly synthesized mediators of inflammation. The molecular mechanisms by which aggregation of the receptors generate these cellular responses are the central focus of our studies. During the past year we focussed on three principal areas: 1) Developing improved methods for discovering proteins other than the subunits of the receptor which might be associated with the receptor either prior to or as a consequence of aggregation. 2) Exploring various aspects of how aggregation may initiate a biochemical cascade, in in vitro systems. 3) Devising a procedure that would permit an assessment of the lifetime of activated receptors. 4) Exploring the role of protein kinase C in the phosphorylation of the receptor. Our new results show: 1) Using chemical crosslinking reagents substantial quantities of other cellular proteins (including the src-like kinase lyn) appear to be associated with the receptor. Aggregation of the receptors prior to crosslinking does not lead to substantial differences in the pattern of associated proteins. 2) Conditions have been developed that allow one to observe in vitro a) Substantially enhanced tyrosine kinase activity in receptors that had been aggregated in vivo but only so long as the receptors remain aggregated, b) Evidence that the in vitro phosphorylation of the receptors occurs via a transphosphorylation. 3) Contrary to the proposals of others, aggregated receptors have a substantial half-life of activation. 4) Protein kinase C is implicated in the phosphorylation of the gamma but not of the beta chain of the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41040-21 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Various Cytotoxic Drug Programs in Diffuse Lupus Nephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry Metzger, M.D.	Acting Chief, Cellular Immunology Section	ARB/NIAMS
	Mark Gourley, M.D.	Special Volunteer	ARB/NIAMS
	Dorothy Scott, M.D.	Special Volunteer	ARB/NIAMS

COOPERATING UNITS *(if any)*

James E. Balow, Senior Investigator, NIDDK
Howard A. Austin, Attending Nephrologist, Clinical Center

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eighty-two patients have been randomized to receive. The study is closed to participants. Rate of progression to renal failure, requirements for re-treatment, and drug toxicities are being assessed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41048-14 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bacterial Cell Wall-Induced Arthritis in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R.L. Wilder	Chief	
Y. Du	Visiting Fellow	
E. Goldmuntz	Med. Staff Fellow	
E. Remmers	Sr. Staff Fellow	All IJDS, ARB/NIAMS
P. Mathern	Visiting Associate	
L. Crofford	Med. Staff Fellow	
H.B. Zha	Visiting Associate	
K. Kalogeras	Sr. Staff Fellow	

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD
Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lewis rats are highly susceptible to bacterial cell wall arthritis, but Fischer rats are relatively resistant. We have previously provided data suggesting that this difference was related to blunted hypothalamic-pituitary-adrenal axis (HPA) responsiveness in the Lewis rat. During the past year, we have continued to characterize the neuroendocrine defect in the Lewis rat compared to the Fischer rat and are relating it to the development of inflammation in response to streptococcal cell walls or lipopolysaccharide. In particular, we have continued to characterize corticotropin releasing hormone (CRH) expressed in peripheral tissues. We have demonstrated the presence of high levels in the inflamed joints of Lewis rats that contrasts with the low levels in arthritis-resistant Fischer rats. Our available data suggest that CRH is produced by macrophages and is also delivered to the inflammatory site through the peripheral nervous system.

We have also demonstrated that HPA axis responses to LPS also differ markedly in the 2 rat strains. We are relating these differences in HPA axis function to the expression of various cytokine genes and cyclooxygenase -1 and -2. These latter studies are not yet complete, but we have developed methods to quantitate the expression of cyclooxygenase-1 and -2, which we believe, like CRH, is intimately involved in regulating both inflammatory and HPA axis responses. Both CRH and COX-2 appear to be strongly downregulated by corticosteroids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066-11 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Synovial Tissues from Patients with RA and Related Conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R.L. Wilder	Chief	
E. Goldmuntz	Med. Staff Fellow	
E. Remmers	Sr. Staff Fellow	All IJDS, ARB/NIAMS
P. Mathern	Visiting Associate	
L. Crofford	Med. Staff Fellow	

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD
Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Animal model studies from our lab have indicated that neuroendocrine mechanisms play a major role in regulating inflammatory processes that are operative in chronic proliferative forms of arthritis. We are actively exploring these concepts in patients with rheumatoid arthritis (RA). We have demonstrated high levels of expression of corticotropin releasing hormone (CRH) in synovial fluids and tissues from patients with RA.

We have also demonstrated that cyclooxygenase -1 and -2 are both expressed in rheumatoid synovia, but only Cox-2 is inducible. Interleukin-1 and PMA are potent stimulators of Cox-2, but not Cox-1. Corticosteroids suppress Cox-2, but not Cox-1 expression. These data support the view that Cox-2, like CRH, may mediate signals between the neuroendocrine and inflammatory systems. Dysregulated expression of the polypeptides appears to be associated with inflammatory joint disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41074-06 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Etiology and Pathogenesis of Idiopathic Inflammatory Myopathy in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB/NIAMS
Frederick Miller, M.D.,Ph.D., Special Volunteer, ARB/NIAMS
Lori Love, M.D.,Ph.D., Special Volunteer, ARB/NIAMS
Nina Raben, M.D,Ph.D., Visiting Scientist, ARB,NIAMS
Ralph Nichols, Ph.D., Staff Fellow, ARB,NIAMS
Jan Dohlman, M.D., Senior Staff Fellow, ARB,NIAMS
Elizabeth Adams, M.D., Clinical Associate, ARB,NIAMS
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB,NIAMS

COOPERATING UNITS *(if any)*

Dr. Terry O'Hanlan

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.2	5.0	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Idiopathic inflammatory myopathy (polymyositis, dermatomyositis, and related disorders) is a family of inflammatory diseases in which disease-specific autoantibodies occur and for which there is considerable indirect evidence pointing to a viral etiology. We have over the past several years, seen and studied and collected serum, blood, and muscle specimens from well over 450 patients suspected of having myositis. We have collected epidemiologic information on many patients. We have cloned, sequenced, and expressed histidyl-tRNA synthetase HRS, the principal target autoantigen in idiopathic polymyositis and dermatomyositis and are analyzing its structure and promoter. We have extended the analysis of HLA antigens in the sets of myositis patients defined by autoantibodies using the sequence specific oligonucleotide hybridization/PCR method. We have analyzed promoter activity of the HRS gene and are currently investigating translational control of its synthesis. We have successfully obtained high level expression of HRS, purified it, and crystallized it. A sensitive, specific, stable ELISA has been developed and a patent applied for.

In collaboration with Dr. Terry O'Hanlan and Dr. Frederick Miller, we have analyzed T cell receptor usage in the muscle of some patients with myositis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41076-06 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Trials in Idiopathic Inflammatory Myopathies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB/NIAMS
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB,NIAMS
Frederick Miller, M.D.,Ph.D., Special Volunteer, ARB/NIAMS
Elizabeth Adams, M.D., Clinical Associate, ARB/NIAMS

COOPERATING UNITS *(if any)*

Jeanne Hicks, M.D., CC Rehabilitation

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to find a better way to treat steroid-resistant myositis (other than inclusion body myositis), we have carried out a randomized crossover trial of intravenous methotrexate with leukovorin rescue and a combination of methotrexate and azathioprine. Accrual of patients is about two-thirds complete.

We have carried out two extensive retrospective analyses of the therapy of myositis. One, of well over 100 patients on whom thorough records of responses were available, was analyzed for the factors that influence the responses to prednisione, methotrexate, and azathioprine. The other was a close analysis of the responses to steroid and cytotoxic therapy in almost 30 patients with inclusion body myositis, including an analysis of the first ever controlled therapeutic trial in that condition which we carried out over the past several years. These have now been published.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41083-04 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Familial Mediterranean Fever

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel L. Kastner, M.D., Acting Chief, Genetics Section, ARB/NIAMS
Ivona Aksentijevich, M.D., IRTA Fellow, Genetics Section, ARB/NIAMS
Elon Pras, M.D., Visiting Associate, Genetics Section, ARB/NIAMS
Ernesto Levy, M.D., Visiting Associate, Genetics Section, ARB/NIAMS
Leandrea Prosen, Biologist, Genetics Section, ARB,NIAMS

COOPERATING UNITS *(if any)*

Heller Institute for Medical Research
Sheba Medical Center
Tel-Hashomer 52621 Israel

Dept Cytogenetics/Molecular Genetics
Adelaide Children's Hospital
North Adelaide, South Australia 5006

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Familial Mediterranean fever (FMF) is a rheumatic disease characterized by intermittent bouts of fever with abdominal pain, pleurisy, and/or arthritis; some patients also develop systemic amyloidosis, leading eventually to renal failure. FMF is inherited as a single autosomal recessive gene (designated MEF), but the biochemical lesion is unknown. The purpose of this project has been to identify the FMF gene by the method of positional cloning. By the beginning of the current year, we had succeeded in mapping the gene causing FMF to the short arm of chromosome 16.

During 1992-3 we have considerable refined the genetic mapping of MEF. After examining 12 genetic markers from distal chromosome 16p, we have narrowed the candidate region to the interval between D16S63 and D16S246, a genetic distance of 3.4 cM (sex-averaged). Moreover, we have identified a highly informative new microsatellite polymorphism at D16S63 that allows rapid haplotyping of FMF families with small amounts of DNA. To define genetic distances in this region more precisely, we have also typed the CEPH reference families for this new microsatellite, as well as D16S80, D16S246, and D16S283. Among our 51 non-Ashkenazi Jewish FMF families there are at least 4 recombinants on the centromeric end of the D16S63-D16S246 interval, indicating the potential to narrow the region of interest even more. We found that FMF in Arabs maps to the same interval, thus increasing the potential pool of informative families.

We have used two additional strategies aimed at localizing MEF: linkage disequilibrium mapping and homozygosity mapping. Previous studies of linkage disequilibrium at D16S94, D16S283, and D16S291 had indicated a probable founder effect among Moroccan Jewish FMF families, but no allelic associations in other subpopulations. More recent typings for D16S246, which is closer to MEF than the other three markers, does indicate strong allelic associations in all Jewish subpopulations studied. This suggests that MEF is closer to the telomeric side of the D16S63-D16S246 interval. Homozygosity mapping is a novel strategy for mapping recessively inherited disease genes in inbred families. With our large panel of consanguineous families, we did find high levels of homozygosity in affected offspring of inbred marriages for a number of loci on chromosome 16p. However, we found this strategy to have limited utility for refined mapping, because of the high gene frequency for FMF, and the relatively low heterozygosities of markers in the region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41084-04 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Features of Keratin and Related Intermediate Filaments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter M. Steinert, Chief, LSB/NIAMS
Others:
John G. Compton, Senior Staff Fellow, LSB/NIAMS
William Idler, Chemist, LSB/NIAMS
James W. Mack, Assistant Professor, Dept of Biochemistry, Howard U Med Sch
Lyuben Marekov, Visiting Scientist, LSB/NIAMS
Jun-Mo Yang, Visiting Fellow, LSB/NIAMS

COOPERATING UNITS *(if any)*

Kathleen Green, Associate Professor, Dept of Pathology, Northwestern U Med Sch; Robert D. Goldman, Professor and Chairman, Dept of Cell, Molecular & Structural Biology, Northwestern U Med Sch; David A. Parry, Professor, Dept of Physics & Biophysics, Massey University

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure, function and expression of the kertin intermediate filaments of human and mouse skin, and the related intermediate filament proteins of other cell types, are being investigated. These studies are designed to understand the structural features that determine how the rod domains of the chains pack to form the filament core. We have used cross-linking studies to determine molecular lengths and the nearest neighbor molecular alignments in the filaments. An assay involving synthetic peptides has been designed to explore the features of the filament models. The glycine-rich end domains of especially the keratin 1/10 filaments of epidermal cells are unique in biology. We believe these organize into a glycine-loop configuration. Current studies are designed to determine how these are packed and how they might interact with other macromolecules coexpressed in epidermal tissues. The glycine-loop sequences on the human keratin 10 chain are extraordinarily polymorphic in size and sequence. Using genomic clones to the human keratin chains 1 and 10, transgenic mice have been constructed to examine the expression characteristics of the genes as well as to probe in vivo the likely functions of the various portions of the chains, such as rod domain segments and glycine-rich end domains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41085-04 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression, Structure and Function of Filaggrin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter M. Steinert, Chief, LSB, NIAMS
Others
John G. Compton, Senior Staff Fellow, LSB, NIAMS
Song Qing Gan, Visiting Associate, LSB, NIAMS
Bernard Korge, Visiting Associate, LSB, NIAMS
James W. Mack, Assistant Professor, Dept of Biochemistry, Howard U. Med Sch
Lyuben Marekov, Visiting Scientist, LSB, NIAMS
Nedialka Markova, Visiting Associate, LSB, NIAMS
Shyh-Ing Jang, IRTA Fellow, LSB, NIAMS

COOPERATING UNITS *(if any)*

Dietmar Mischke, Staff Scientist, Free Univ. of Berlin, Germany; Howard University, Washington, D.C.

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Filaggrin is a major product of terminally differentiating mammalian epidermal cells that is thought to be involved in the aggregation and specific alignment of keratin intermediate filaments during the final stages of differentiation. Thus, filaggrin is an important example of an intermediate filament-associated protein. We have isolated both cDNA and genomic clones which show that filaggrin is expressed initially as a large polyprotein precursor, profilaggrin, which is subsequently proteolytically processed into individual functional filaggrin molecules. The structure of the gene for human profilaggrin has now been settled; it consists of 3 exons separated by 2 introns, the first of which is huge (about 10 kbp). The amino-terminal end of human and mouse profilaggrins possess two functional calcium-binding domains of the EF-hand type. There is evidence for multiple alternate splicing of introns and exons, giving rise to a series of other calcium-binding proteins of as yet unknown function. We have constructed genomic clones for the production of transgenic mice. We have begun a systemic analysis of regulatory sequences that control the expression of the profilaggrin gene system. We have determined that filaggrin aggregates keratin and vimentin intermediate filaments by ionic interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41086-04 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression, Structure and Function of Loricrin, a Major Cell Envelope Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Peter M. Steinert, Chief, LSB/NIAMS
Kozo Yoneda, Visiting Fellow, LSB/NIAMS
Eleonara Candi, Special Volunteer, LSB/NIAMS
James Mack, Special Volunteer, LSB/NIAMS

COOPERATING UNITS *(if any)*

Genaro Melino, Professor, Dept of Pathology, University Tor Vergata, Rome; Howard University, Washington, D.C.

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cornified cell envelope constitutes a multi-component 15 nm thick layer of highly insoluble protein on the inside of the plasma membrane of terminally differentiated epithelial cells. Based on a variety of data, we now know that loricrin is a major component of the epidermal cell envelope. It is crosslinked to the envelope by way of N^{ϵ}-(γ-glutamyl)lysine isodipeptide crosslinks, catalyzed by the action of transglutaminases. Loricrins are glycine-rich proteins that contain the highly flexible glycine loop motif. We have expressed human locricrin in a baculovirus system to study its biochemical properties and the mechanism(s) by which it is crosslinked. In order to study its precise role in the epidermis, and the regulation of its expression, we have used transgenic technology. A construct containing the full-length human gene and flanking sequences can direct the proper site- and stage-specific over-expression of human loricrin in transgenic mice. Since the mice are normal, loricrin is presumably a very late, if not the last, component to be added to the cell envelope.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41087-04 LSB

PERIOD COVERED

October 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidermal Transglutaminases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter M. Steinert, Chief, LSB/NIAMS
Others:
Soo-Youl Kim, Visiting Fellow, LSB/NIAMS
Kozo Yoneda, Visiting Fellow, LSB/NIAMS
Seung-Chul Lee, Visiting Fellow, LSB/NIAMS
Jeung-Hoon Lee, Visiting Fellow, LSB/NIAMS
Edit Tarcsa, Visiting Fellow, LSB/NIAMS
In-Gyu Kim, Associate Professor, Dept of Biochemistry, Inha U Med School

COOPERATING UNITS *(if any)*

Soo-II Chung, Senior Investigator, LCDO/NIDR;O. Wes McBride, Senior Investigator, LB/DCBDC/NCI; Sang-Chul Park, Professor, Dept of Biochemistry, Seoul National University; Inha University

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 3.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transglutaminases from isodipeptide crosslinks between acceptor amide groups of glutaminyl residues and donor epsilon-NH2 group of lysines. In the epidermis, and other stratified squamous epithelial tissues, these enzymes are thought to be involved in the crosslinking of putative protein components to form the insoluble cell envelope. Using molecular biology approaches, we have found that there are 3 different transglutaminase activities in normal human and mouse epidermis. These are known as the K(TGase1), C(TGase2) and E(TGase3) enzymes. We have isolated and characterized cDNA clones encoding full-length TGase1 and TGase3 systems. The complete genomic structure (14.1kbp) of the TGase1 systems has been completed. Work is in progress on the genomic structure of the TGase3 gene. Full length and deletion constructs of the TGase1 and TGase3 enzymes have been expressed in E. coli and in mammalian cells in an effort to understand the structural/functional domains. The aim of all of these studies is to determine the likely functions of these different activities in normal epidermis, and whether or how these may be involved in pathology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41088-03 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Neuroendocrine Factors in the Autoimmune Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R.L. Wilder	Chief, IJDS	
P. Mathern	Visiting Associate	
L. Crofford	Med. Staff Fellow	All IJDS, ARB/NIAMS
K. Kalogeras	Sr. Staff Fellow	

COOPERATING UNITS *(if any)*

C. Amos and S. Doyle, Laboratory Skin Biology, NIAMS
F. Miller and A. Mbauya, CBER, FDA
Developmental Endocrinology Br. NICHD

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies from animal models have suggested the possibility that hypothalamic-pituitary-adrenal axis and other neuroendocrine dysfunctions may play a role in rheumatoid arthritis and other autoimmune conditions. These animal studies have also suggested that HPA axis dysfunction may predispose an individual to many different types of autoimmune disease. For these reasons, we have initiated studies to evaluate HPA axis function and familial clustering of autoimmune diseases in the families of patients with rheumatoid arthritis and related conditions. Our initial studies support the hypothesis that HPA axis responsiveness is blunted in rheumatoid arthritis. Over the past year, we have continued to collect data, but the patient recruitment, speciman collection and analysis are not complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41089-02 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Studies of Hereditary Skin Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: S Bale, Acting Chief, GSS, LSB

Others:

C Amos, Sr. Staff Fellow, GSS,LSB,NIAMS
A Revell, Biologist, GSS,LSB,NIAMS
P Steinert, Branch Chief, LSB,NIAMS
B Korge, Special Volunteer, LSB,NIAMS
N Markova, Visiting Assoc, LSB,NIAMS
V Kimonis, Clinical Associate, CC
S Doyle, Research Nurse,GSS,LSB,NIAMS
L Russell, Sr. NRC Fellow
J Compton, Sr. Staff Fellow, LSB,NIAMS
C Chipev, Visiting Assoc,LSB,NIAMS
G Richard, Dermatologist, Erfurt Univ. Germany

COOPERATING UNITS *(if any)*

J DiGiovanna, Expert,DB,DCBC,NCI; OW McBride, Section Chief, LSB,DCBC,NCI; A Bale, Asst Professor, Yale University, Depts of Dermatology and Genetics, New Haven, CT; EEB,DCE,NCI; A Goldstein, Sr. Staff Fellow, EEB, DCE, NCI; Erfurt University,

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	2.6	1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the genetic basis of a variety of skin disorders, including six disorders of cornification (epidermolytic hyperkeratosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, Darier's disease, Hailey-Hailey disease, and ichthyosis vulgaris) and two disorders predisposing to skin cancer (basal cell nevus syndrome, familial malignant melanoma). Patients and their families are recruited for study. They travel to the NIH clinics (or rarely we travel to them) to enable our research group to perform detailed family and medical histories, skin examinations, skin biopsies, phlebotomy, and clinical photography. DNA is extracted from patient's blood and permanent cell lines established. Skin samples are used to confirm diagnoses and to investigate ultrastructural abnormalities specific to each disease. Clinical heterogeneity (different clinical appearances of the "same" disease) is investigated using the information collected. DNA-based polymorphisms (i.e. RFLPs, PCR) are used for linkage studies to determine the chromosomal location of the skin disease locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41090-02 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of New Statistical Methods for Genetic Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-P.I.: SJ Bale, Acting Chief	Genetic Studies Section	LSB/NIAMS
Co-P.I.: C Amos, Staff Fellow	Genetic Studies Section	LSB/NIAMS

COOPERATING UNITS *(if any)*

Howard University, Washington, D.C.

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases. We have worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41091-02 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Consultation for Genetic Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Chris Amos	Staff Fellow	GSS/LSB/NIAMS
Others:		
Sherri J Bale	Acting Chief	GSS/LSB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New statistical methods for analysis of the genetic component of complex familial diseases are needed in order to determine the relative contribution of environment and heredity to a variety of traits, and to elucidate the specifics of these determinants. We have been focussing on the problem of genetic linkage studies in complex diorders (e.g. arthritis, other autoimmune diseases, mitochondrial diseases) when certain usual conditions of analysis are not met (e.g. known mendelian model, large sample size, non-normally-distributed quantitative trait measurements).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41092-02 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mapping of Genes and Genetic Polymorphisms in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R.L. Wilder	Chief, IJDS	
Y. Du	Visiting Fellow	
E. Goldmuntz	Med. Staff Fellow	
E. Remmers	Sr. Staff Fellow	All IJDS, ARB/NIAMS
P. Mathern	Visiting Associate	
L. Crofford	Med. Staff Fellow	
H.B. Zha	Visiting Associate	

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rats are an important experimental model for many human diseases, many of which have a genetic component. As followup of our previous and ongoing work demonstrating impressive differences in the phenotypic responses of LEW and F344 rats to proinflammatory and noninflammatory stimuli, we are developing a genetic linkage map for rats, specifically applicable to these rat strains. We have now identified 250 polymorphisms and have mapped about 200 of these to 17 of 20 rat chromosomes using an F2 intercross of F344 x LEW parents. We do not yet have markers for 3 rat chromsomes. We have demonstrated impressive conservation of synteny between rat, mouse and human chromosomes. We have also extended our efforts to identify polymorphisms to 13 additional inbred strains of rats. Importantly, we have identified approximately an additional 150 polymorphic markers that differ between the LEW and inbred BN rats, another relatively autoimmune disease resistant rat strain. In summary, we have markedly increased our ability to chromosomally localize genes that control important phenotypic traits in rats. Moreover, these markers are highly useful for genetic monitoring of inbred rat strains.

As part of our effort to localize the athymic nude gene in the rat, we have genomically cloned, sequenced and mapped the rat epimorphin gene to chromosome 12 and have excluded it as a candidate locus for the rat athymic nude trait, which has been localized to chromosome 10.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41093-02 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression, Structure and Function of Trichohyalin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter M. Steinert, Chief, LSB/NIAMS
Seung-Chul Lee, Visiting Fellow, LSB/NIAMS
In-Gyu Kim, Associate Professor, Dept of Biochemistry, Seoul Nat'l U Med Cnt
Others:
Lyuben Marekov, Visiting Scientist, LSB/NIAMS

COOPERATING UNITS *(if any)*

Soo-Il Chung, Senior Investigator,LCDO/NIDR; O. Wesley McBride, Senior Investigator, LB/DCBDC/NCI; University of North Carolina

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Trichohyalin is a major differentiation product of the inner root sheath cells of the hair follicle, where it is initially deposited in large dense granules, termed trichohyalin granules. Subsequently, during terminal differentiation, it is dispersed and becomes associated with the keratin intermediate filaments of the inner root-sheath cells. Thus it seems to serve largely as an intermediate filaments associated protein in this tissue. It is also expressed in the medulla of coarser hairs where it does not interact with filaments, but rather changes into a loose amorphous product. Trichohyalin is of interest because it is a major substrate for transglutaminases, and it also undergoes postsynthetic modifications of certain of its arginine residues to citrullines. Of especial interest is the recent observation that trichohyalin is also expressed in the epidermis, but its role in epidermal differentiation and role in pathology remain to be elucidated. We have obtained a cDNA clone by PCR analysis of genomic DNA and have used this as a probe to isolate the human trichohyalin gene. The human trichohalin protein consists largely of a series of quasi-repeating peptides, and interestingly like profilaggrin, its amino terminus contains two functional calcium-binding domains of the EF-hand type. Our interest in this system is to explore its role as a transglutaminase substrate, as a major calcium-binding protein, as an important new intermediate filament associated protein, and its role, if any, pathology.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41094-02 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Bases of Hereditary Skin Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter M. Steinert, Chief, LSB/NIAMS
Sherri J. Bale, Geneticist, LSB/NIAMS
Constantin C. Chipev, Visiting Associate, LSB/NIAMS
Others: John G. Compton, Senior Staff Fellow, LSB/NIAMS
William W. Idler, Chemist, LSB/NIAMS
Bernard P. Korge, Visiting Associate, LSB/NIAMS
Nedialka Markova, Visiting Associate, LSB/NIAMS
Gabriele Richard, Staff Physician, Erfurt U Med Sch
Jun-Mo Yang, Special Volunteer, LSB/NIAMS

COOPERATING UNITS *(if any)*

John D. DiGiovanna, Expert, DB/DCBCD/NCI; O. Wesley McBride, Senior Investigator, LB/DCBDC/NCI; University of Erfurt, Germany,

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization

INSTITUTE AND LOCATION

NIAMS, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a number of heritable diseases of cornification of human epidermis, which affect the suprabasal cell layers of the epidermis. These include the various ichthyoses (such as ichthyosis vulgaris, lamellar ichthyosis, bullous- and nonbullous congential ichthyosiform erythroderma) and others including Darier's disease, Hailey-Hailey disease, etc. In all cases, these diseases follow simple or relatively simple genetic expression characteristics implying they involve simple mutations in one (or more) proteins expressed in epidermal cells committed to terminal differentiation. Although some morphological and biochemical analyses have been performed on several of these, in no case has the underlying genetic defect been identified. This project involves a collaboration with the primary genetic studies work of Dr. Bale (see project number Z01 AR 41089-02). Following identification of suitable families, linkage analyzes will be performed using PCR mapping of polymorphisms of candidate and other gene products of known location, to be followed by identification of the specific mutations in the genes that cause these occurrences of the disease. To date, we have identified a total of 10 mutations in either the keratin 1 - keratin 10 chains in separate families with epidemiolytic hyperkeratosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41095-02 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Translational and Rotational Power Terms in 6 Degree-of-Freedom Joint Modeling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

F.L. Buczek	Staff Fellow, Biomechanics Laboratory	ARB/NIAMS
J.P. Holden	IRTA Fellow, Biomechanics Laboratory	ARB/NIAMS

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)
S.J. Stanhope-Dir., Biomechanics Lab (BML) RMD/CC
T.M. Kepple-Programmer Specialist & K.L. Siegel-Senior Staff Fellow, BML RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An engineering methodology called "inverse dynamics" provides the resultant forces & moments at major anatomical joints during walking. These "joint reactions" perform mechanical work, & consequently have power associated with them. Joint powers have been used to evaluate mechanical energy flows for individual joints. If added for all major joints of the body, the sum represents a mechanical energy cost for movement, which is an important parameter for estimation of mechanical efficiency. Traditionally, joint models have allowed only rotations at joints, without accounting for translations such as sliding & compression/distraction. Our immediate objective is to implement full six degree-of-freedom (DOF) joint models for the lower extremity, allowing for three rotational & three translational movements (DOFs). Traditional models assumed that translational powers canceled each other across a joint; our new methods include joint powers associated with the three translational DOFs. We believe this detailed model will provide more robust calculations for joint powers, improving reliability & accuracy in total mechanical power estimates. Lower extremity data are collected over full stride cycles for multiple walking trials. Ensemble averages & coefficients of variability are determined for each DOF.

Results at the ankle joint for an intra-subject analysis (n=5) showed that X-axis rotational power (associated with ankle dorsi/plantarflexion) predominates, with a peak value of 360 W. The next largest peak in joint power was for the vertical translational DOF, and was less than 10% of the predominant peak. Positive work during push-off was significantly less ($p \leq 0.05$) for the 6 DOF model (27.9 J) than for either 1 or 3 DOF models (30.3 & 29.9 J, respectively). Negative work during early stance was also significantly less for the 6 DOF model (-10.3 J) than for either 1 or 3 DOF models)-13.1 & -12.6 J, respectively). Inter-subject analyses (n=50) were conducted on joint power data, with similar results. Joint powers at the knee are currently being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41099-02 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Genetic Basis for Metabolic Myopathies.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB,NIAMS
Nina Raben, M.D.,Ph.D., Visiting Scientist, ARB,NIAMS
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB,NIAMS
Cornelius Boerkoel, III, M.D.,Ph.D., IRTA, ARB,NIAMS
Frederick Miller, M.D.,Ph.D., Special Volunteer, ARB,NIAMS
Elizabeth Adams, M.D., Clinical Associate, ARB,NIAMS
Catherine Nicastri, Biologist, ARB,NIAMS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of mutations and correlation with clinical illness has been greatly expanded by the analysis of a number of families with both phosphofructokinase (PFK) and acid maltase deficiencies. Plans for gene therapy of acid maltase deficiency have moved forward in several areas.

A number of metabolic/genetic myopathies have been diagnosed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41100-01 ARB

PERIOD COVERED

March 1, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Skeletal Tracking with Skeletal Fixation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.P. Holden IRTA Fellow, Biomechanics Laboratory ARB/NIAMS

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)
S.J. Stanhope Dir., Biomechanics Lab (BML) RMD/CC
K.L. Siegel-Senior Staff Therapist & T.M. Kepple-Programmer Specialist, BML RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Kinematic gait analysis is increasingly used for a wide variety of purposes, including clinical evaluation to aid surgical decision-making and the study of joint and limb motion for the design of prostheses and braces. Standard gait analysis procedures currently involve the estimation of skeletal motion by tracking the movement of targets placed on the skin. The motion of the skin and underlying soft tissues relative to the bone is known to be a source of error in the measurement of skeletal motion, but the magnitude and pattern of the soft tissue motion has not been reported in the literature. The purpose of this project is to determine the degree and pattern of error introduced into standard kinematic gait analysis procedures by soft tissue motion around the femur and shank. A critical initial phase of this project is the development of a relatively safe, simple, and reproducible method for attaching and tracking targets fixed to bone.

Measurement of actual bone movement is accomplished using a percutaneous skeletal tracker (PST), a device designed to achieve skeletal fixation of targets. A PST is attached to the distal femur and distal shank; surface mounted targets are also attached using standard procedures. A three-dimensional target tracking system measures the movement of both target sets as subjects walk in the laboratory. The six degree-of-freedom motions of the shank and thigh are calculated using each target set, and the difference in motions represents the error introduced into the measurements by the soft tissues.

Three subjects have undergone PST placement and data collection without complications. Rigid PST fixation to the distal shank was achieved for each subject. Fixation was not sufficient at the distal femur, however, but several important questions concerning pin placement at the knee were addressed. The skeletal motion data collected for the shank are currently being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41102-01 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Cystinuria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel L. Kastner, M.D., Ph.D., Acting Chief, Genetics Section, ARB/NIAMS
Elon Pras, M.D., Visiting Associate, Genetics Section, ARB/NIAMS

COOPERATING UNITS *(if any)*

Heller Institute for Medical Research
Sheba Medical Center
Tel-Hashomer 52621 Israel

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cystinuria is an autosomal recessive disease in which there is excessive excretion of cystine in the urine, with precipitation of urinary cystine stones. Patients with this disorder may have renal colic, urinary tract obstruction, secondary urinary tract infections, and, if untreated, renal insufficiency. The chromosomal location of the cystinuria gene and the biochemical basis of cystinuria are unknown. The purpose of this project is to identify the chromosomal location of the cystinuria gene.

During the first year of this project we have obtained peripheral blood samples from 9 cystinuria families (52 individuals, 22 affected). In most cases Epstein-Barr virus-transformed B cell lines were established. Genomic DNA was isolated from cell lines and/or freshly isolated lymphocytes.

We began our genetic studies by testing for linkage to chromosome 16, since we had observed a Libyan Jewish family in which cystinuria co-segregated with familial Mediterranean fever (encoded on chromosome (16p). Using the larger panel of families and a group of highly informative microsatellite markers, we were unable to confirm linkage of the cystinuria gene to the short arm of chromosome 16.

We have subsequently begun a systematic search of the genome to identify the chromosomal location of the cystinuria gene. After having examined approximately 40 additional markers, we do not yet have conclusive evidence placing the cystinuria gene on a specific chromosome. During the next year we plan to continue this search, examining markers spaced at 20 cM intervals throughout the genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41103-01 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel L. Kastner, M.D.,Ph.D., Acting Chief, Genetics Section,ARB/NIAMS
Michael McDermott, M.D., Visiting Scientist, Genetics Section,ARB/NIAMS

COOPERATING UNITS *(if any)*

Department of Rheumatology University College Cork, Ireland	Department of Human Genetics University of Utah

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rheumatoid arthritis (RA) is a chronic peripheral arthritis of unknown etiology affecting 1-2% of the world's population. Studies of identical twins and multiplex families strongly suggest a genetic component of RA susceptibility, but these same studies point to multiple genes and reduced penetrance. The purpose of this project is to identify the chromosomal location of these genes.

Population studies have already shown that at least one susceptibility locus is probably associated with the HLA-DR4 and DR1 alleles of the major histocompatibility complex (MHC). Since HLA-DR molecules are known to present antigen to the T-cell receptor (TCR), we speculated that TCR genes might also contribute to RA susceptibility. In the collagen-induced arthritis mouse model of RA, disease susceptibility is jointly determined by MHC and TCR genes. We therefore decided to study the role of TCR genes in determining RA susceptibility in man.

DNA specimens were obtained from multiplex RA families seen in rheumatology clinics in Cork (Ireland), Salt Lake City, and Toronto. A total of 259 individuals were studied (77 affected). Families were genotyped for markers associated with the TCR beta and gamma chains, both by Southern blotting and by the polymerase chain reaction. Data were analyzed by a linkage analysis method that allowed for more than one susceptibility locus, and by affected sib pair analysis.

To date we have obtained suggestive, although not conclusive evidence that the TCR beta chain gene may encode RA susceptibility. Using the Vbeta6.7 PCR marker in affected sib pair analysis, there was more sharing of haplotypes than expected by chance. The p value or this comparison was 0.0., but the value required for significance is controversial (it may be as low as 0.001). We have found no evidence that the TCR gamma gene encodes RA susceptibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41104-01 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies of the Rheumatic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.H. Klippel Clinical Director, NIAMS
F.J. Stafford Senior Staff Fellow, ARB
M. Gourley Senior Staff Fellow, ARB
D. Scott Volunteer, ARB

COOPERATING UNITS (if any)

C. Yarboro Nursing; CC
F. Pucino Pharmacy, CC
T. Fleisher Clinical Immunology, CC

LAB/BRANCH

ARB

SECTION

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have conducted a series of clinical investigations involving patients with systemic lupus erythematosus - a chronic, relapsing and remitting disorder characterized by immune-mediated inflammation. The current studies have mainly focused on patients with lupus glomerulonephritis in an attempt to assess practical issues related to therapy with the alkylating agent cyclophosphamide and, in particular, to investigate new approaches to disease management. A feasibility study has demonstrated that outpatient therapy with bolus oral cyclophosphamide is safe and effective and an improved regimen for minimizing cyclophosphamide-induced nausea has been studied. A small pilot study of a murine monoclonal anti-CD5 ricin A chain immunoconjugate in 6 patients documented a chronic reduction in peripheral T cell populations with evidence of clinical improvement in two patients with lupus nephritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41096-02 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nonequilibrium in the Translational Equations of Motion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

F.L. Buczek	Staff Fellow, Biomechanics Laboartory	ARB/NIAMS

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

S.J. Stanhope	Dir., Biomechanics Lab (BML)	RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was inactive during the period covered.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41097-02 ARB

PERIOD COVERED

October 1, 1992 to February 12, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Changes in Ankle Function After Forefoot Arthroplasty in Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

F.L. Buczek	Staff Fellow, Biomechanics Laboratory	ARB/NIAMS

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was inactive during the period covered.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

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Z01 AR 41098-02 ARB

PERIOD COVERED

October 1, 1992 to February 12, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Study of Falling in Patients with Muscle Weakness Due to Myositis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

F.L. Buczek Staff Fellow, Biomechanics Laboratory ARB/NIAMS

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH

Arthritis and Rheumatism Branch

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  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was inactive during the period covered.

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